Progression of HIV-1 infection

Monitoring of HIV-1 DNA in peripheral blood mononuclear cells by PCR


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Summary. We present data on the distribution of human immunodeficiency virus (HIV-1) proviral DNA in different subsets of peripheral blood mononuclear cells (PBMCs) over an observation period of eight months. Eleven patients with well documented HIV-1 infection were studied. The PBMCs were obtained at two intervals and purified by fluorescence-activated cell sorting (FACS) after staining with FITC-labelled monoclonal antibodies. Varying numbers of FACS-sorted CD4+ cells, CD8+ cells and peripheral monocytes were assayed for HIV-1 proviral DNA (env and gag region) by PCR. Samples from patients at CDC stages II or III had to contain $10^3$–$10^4$ cells in order to allow detection of proviral HIV-1 DNA. At CDC stage IV, however, HIV-1 DNA was detected in as few as 100 CD4+ T-lymphocytes. In contrast, in peripheral monocytes HIV-1 DNA was not regularly found. CD8+ cells did not harbor detectable amounts of proviral DNA.

During an observation period of eight months, the rate of infected CD4+ T-lymphocytes increased significantly in three patients while staying constant in the remaining eight patients. This increase of the infection rate was paralleled by clinical progression in one patient and by a decrease of the absolute number of CD4+ cells in another patient. The percentage of CD4+ cells harboring the viral genome increases in the course of the disease. These results may help to explain the decrease in CD4+ T-lymphocyte counts during HIV-1 infection.

Introduction

HIV-1 can be isolated from the CD4+ lymphocytes of infected subjects [8]. Besides, virus isolation is possible from monocytes expressing the CD4 molecule [14]. CD8+ cells, however, do not express this receptor and cannot be infected with HIV-1 [10]. Most attempts to identify viral proteins in single blood cells by immunostaining methods have failed. This is generally explained by the low
number of peripheral blood mononuclear cells (PBMCs) showing virus replication [3, 15]. Proviral DNA of HIV-1, in contrast, may be detectable in latently infected cells expressing neither viral RNA nor viral proteins. The detection of low numbers of proviral DNA molecules has become possible since the advent of in vitro DNA amplification [12]. The presence of gag or env genes of HIV-1 has been reported even in seronegative subjects [4, 6, 12]. PCR studies have shown that in the peripheral blood HIV-1 DNA resides preferentially in the CD4+ cells [5, 15] where it is mainly found integrated into the cellular genome as proviral DNA [13]. Asymptomatic seropositive persons have lower numbers of cells harboring HIV-1 DNA than AIDS patients, where possibly all CD4+ cells may be infected during the course of the disease. HIV-1 DNA could be detected in as few as 100 CD4+ lymphocytes using PCR with 35 amplification cycles [5]. Since clinical progression of HIV-1 infection might be associated with a heavier burden of integrated viral DNA, we have carried out an eight months longitudinal study on eleven patients with HIV-1 infection in order to correlate our laboratory data with the clinical course.

Materials and methods

Patients
The 11 patients presented in this study were promiscuous homosexual men, 18–35 years old, with long standing HIV-1 infection, who had been admitted to our hospital. None of the patients had received AZT or other antiviral compounds. Clinical assessment according to the CDC classification had been performed during regular visits of the patients in the outpatient department.

Preparation and purification of PBMCs
Mononuclear cells were isolated from citrate anticoagulated peripheral blood which had been diluted 1:2 with phosphate buffered saline (PBS). PBMCs were purified by Ficoll-Paque (Pharmacia), washed twice in PBS and subjected to plastic adherence. Sorting was carried out using the FACStar Plus cell sorter (Becton Dickinson, Mountain View, CA, U.S.A.) equipped for four parameter analysis as described [5]. Cells were sorted on the basis of forward and sideward light scatter fluorescence (CD4+: FITC+; CD8+: Phycoerythrin+; monocytes/macrophages: FITC− and Phycoerythrin−; Becton Dickinson, Mountain View, CA, U.S.A.) after immunostaining and fixation [2]. Reanalysis of the sorted cells revealed a purity of >99% for CD8+ cells, >99% for CD4+ cells, and >98% for monocytes/macrophages. CD8+ were contaminated by <0.3% CD4+ cells and vice versa. Monocytes/macrophages contained <0.5% T cells. At least 10^5 cells of each subset (CD8+ cells, CD4+ cells and monocytes) were isolated for each experiment.

DNA-preparation from PBMCs
After separation and sorting, cells were washed in PBS. 1 × 10^6–1 × 10^7 cells were resuspended in 500 μl lysis buffer (10 mM Tris, 10 mM Na-EDTA, 50 mM NaCl, 0.5% SDS, 1 mg/ml proteinase K, pH 7.6). After an incubation period of 2 h at 60°C, proteinase K was inactivated at 95°C (10 min). The crude DNA extract obtained by this procedure was used for PCR.